Vaccine 33 (2015) 3801–3812

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Review

Three randomized trials of maternal influenza immunization in Mali, Nepal, and South Africa: Methods and expectations

Saad B. Omer[a,b,c,d,*], Jennifer L. Richards[b], Shabir A. Madhi[e,f,g], Milagritos D. Tapia[h,i], Mark C. Steinhoff[j,k], Anushka R. Aqil[a], Niteen Wairagkar[l], for the BMGF Supported Maternal Influenza Immunization Trials Investigators Group

[a] Hubert Department of Global Health, Emory University Rollins School of Public Health, Atlanta, GA, USA
[b] Department of Epidemiology, Emory University Rollins School of Public Health, Atlanta, GA, USA
[c] Emory Vaccine Center, Atlanta, GA, USA
[d] Department of Pediatrics, Emory University School of Medicine and Children's Healthcare of Atlanta, Atlanta, GA, USA
[e] Medical Research Council, Respiratory and Meningeal Pathogens Research Unit, Swindon, UK
[f] Department of Science and Technology—National Research Foundation, Vaccine-Preventable Diseases, Johannesburg, South Africa
[g] National Institute for Communicable Diseases, The National Health Laboratory Service, Centre for Vaccines and Immunology, Johannesburg, South Africa
[h] Centre pour le Développement des Vaccins, Bamako, Mali
[i] University of Maryland School of Medicine, Center for Vaccine Development, Baltimore, MD, USA
[j] Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA
[k] Cincinnati Children's Hospital Global Health Center, Cincinnati, OH, USA
[l] Bill & Melinda Gates Foundation, Seattle, WA, USA

ARTICLE INFO

Article history:
Received 6 December 2014
Received in revised form 5 May 2015
Accepted 27 May 2015
Available online 19 June 2015

Keywords:
Influenza
Maternal immunization
Pooled analyses
Mali
South Africa
Nepal

ABSTRACT

Influenza infection in pregnancy can have adverse impacts on maternal, fetal, and infant outcomes. Influenza vaccination in pregnancy is an appealing strategy to protect pregnant women and their infants. The Bill & Melinda Gates Foundation is supporting three large, randomized trials in Nepal, Mali, and South Africa evaluating the efficacy and safety of maternal immunization to prevent influenza disease in pregnant women and their infants <6 months of age. Results from these individual studies are expected in 2014 and 2015. While the results from the three maternal immunization trials are likely to strengthen the evidence base regarding the impact of influenza immunization in pregnancy, expectations for these results should be realistic. For example, evidence from previous influenza vaccine studies – conducted in general, non-pregnant populations – suggests substantial geographic and year-to-year variability in influenza incidence and vaccine efficacy/effectiveness. Since the evidence generated from the three maternal influenza immunization trials will be complementary, in this paper we present a side-by-side description of the three studies as well as the similarities and differences between these trials in terms of study location, design, outcome evaluation, and laboratory and epidemiological methods. We also describe the likely remaining knowledge gap after the results from these trials become available along with a description of the analyses that will be conducted when the results from these individual data are pooled. Moreover, we highlight that additional research on logistics of seasonal influenza vaccine supply, surveillance and strain matching, and optimal delivery strategies for pregnant women will be important for informing global policy related to maternal influenza immunization.

1. Introduction

Influenza infection in pregnancy can adversely impact maternal, fetal, and infant outcomes [1–7]. While pregnant women tend to be infected with the influenza virus at similar rates as non-pregnant women of similar socio-demographic characteristics, pregnancy increases their likelihood of adverse outcomes after influenza infection. There are physiological changes in pregnancy such as decreased lung capacity, lower tidal volume, and high cardiac output that could play a role in increasing pregnant women's vulnerability to adverse outcomes after influenza infection [8,9]. More importantly, there are immunological changes in pregnancy, such as Th1 to Th2 shift and attenuated cell mediated immunity that modify a pregnant woman's

* Corresponding author. Tel.: +1 404 727 9814.
E-mail address: somer@emory.edu (S.B. Omer).

http://dx.doi.org/10.1016/j.vaccine.2015.05.077

ability to respond to certain infections—particularly viral infections [10,11].

The Bill & Melinda Gates Foundation (BMGF) is supporting three large, randomized trials evaluating the efficacy and safety of maternal immunization to prevent maternal and young–infant (<6 months of age) influenza disease in Nepal, Mali, and South Africa [10]. The primary results from the South Africa trial have been published [11] and results from the other two trials are expected in 2014/2015 and will advance decisions on influenza vaccine introduction for pregnant women in resource-limited settings. Furthermore, a pooled analysis of data from these trials will be valuable for understanding the benefits of and building the evidence base for this intervention, particularly for outcomes for which individual trials may not have been powered.

Since the evidence generated from these trials will be complementary, in this paper we present a side-by-side description, as well as similarities and differences between these trials in terms of study location, design, outcome evaluation, and laboratory and epidemiological methods. We discuss the expectations from these trials and describe the outcomes selected for pooled analyses, the process and criteria for selecting these analyses, and statistical methods to be used in the analyses. This will serve as a resource for interpreting findings from the three BMGF-sponsored trials as the results from these studies become available in the coming years.

2. Rationale for conducting maternal influenza immunization trials

Influenza vaccination in pregnancy is an appealing strategy to protect pregnant women and their young–infants. There have been several recent developments in the field of maternal influenza immunization. The World Health Organization's Strategic Advisory Group of Experts on Immunization has concluded that vaccination of pregnant women is safe [12]. Furthermore, in a randomized controlled trial in Bangladesh, administration of inactivated influenza vaccine in the third trimester of pregnancy was associated with reduction of confirmed influenza (using rapid ELISA test) by 63% among infants younger than 6 months of age [13]. Maternal influenza immunization has also been associated with protection against adverse birth outcomes such as prematurity and small for gestational age birth in observational studies and post-hoc analyses of trial data [3,14], although this finding has not been consistently observed by others [15] (particularly in studies that do not account for influenza infection/circulation). Consequently, whereas these advances are promising, many questions remain.

While the Bangladesh trial was a significant milestone for developing an evidence base for maternal influenza immunization, it had some limitations. For example, this trial was conducted during a single influenza season over an 11 month period. Since the epidemiology of influenza varies substantially by geography and season, the findings from the Bangladesh trial need to be replicated in other settings and over multiple seasons. Another limitation of this trial is that the efficacy of maternal influenza vaccination was computed in comparison with the pneumococcal polysaccharide vaccine (PPSV). While PPSV served as the comparison group for the influenza vaccine analysis, PPSV was the main intervention when the trial was initiated. PPSV could have affected the risk of non-laboratory-confirmed outcomes such as influenza-like illness, which could have impacted on the true efficacy of maternal influenza immunization against some outcomes. Moreover, the association between maternal influenza immunization and birth outcomes was evaluated post hoc in the Bangladesh trial and has never been evaluated using a priori outcomes in a randomized controlled trial.

3. Rationale for pooled analysis

We sought to conduct a pooled analysis of data from the three trials to further build an evidence base for maternal immunization interventions. While the three trial sites will provide necessary data as it relates to maternal immunization, by pooling the data, we will be able to examine various outcomes for which individual trials may not have been powered. Pooled analysis, often described as meta-analysis of individual level data, has several advantages over "traditional" meta-analysis (i.e. meta-analysis based on summary estimates). In contrast with traditional meta-analysis, pooled analysis allows for better standardization of analytical variables, more robust confounder control, and greater ability to evaluate heterogeneity and effect modification. Therefore, given that we have access to individual level data from the three trials, we opted for the pooled analysis approach rather than using the traditional group-level meta-analysis to synthesize information from these trials.

4. Trial descriptions

A side-by-side description of the three trials is provided in Tables 2–4 and supplement; a comparison of maternal mortality ratios and infant mortality rates is also provided in Table 1. Briefly, all three are randomized, controlled, blinded trials. Enrollment occurred from mid-September 2011 to mid-April 2013 in Mali, mid-April 2011 to mid-April 2013 in Nepal, and March 2011 to August 2011 and March 2012 to July 2012 in South Africa. The enrollment was targeted to coincide with the influenza season in South Africa; whereas, the other two sites enrolled and vaccinated participating women year round. In Nepal, multiple peaks of influenza activity were observed in December 2011, August–October 2012, May 2013, June–August 2013, March–April 2014, July–September 2014, and February–March 2015. In South Africa, the 2011 season had 2 distinct peaks. The first peak, starting the week of 13 June which was followed by a second peak, on the week of 26 September; 2012 season had a peak starting on the week of 20 August. In Mali, peaks were observed in September/October and in February from 2010 to 2014.

In Mali, where there is no formal influenza vaccination policy, pregnant women receiving prenatal care at six referral centers and community health centers in Bamako were offered enrollment. In Nepal, where there is also no formal influenza vaccination policy, women who were or who became pregnant in 9 Village Development Committees in Sarlahi District in southern Nepal were included; the participants were all identified by baseline household surveys. In South Africa, where there has been a national campaign for influenza vaccination of pregnant women since 2010, enrollment was conducted among women accessing prenatal care at Chris Hani-Baragwanath Hospital or at one of four community-based antenatal clinics in Soweto region in Johannesburg. Within the South African program, separate cohorts of HIV-uninfected women ($n = 2108$) and HIV-infected women ($n = 180$) were enrolled. Only the HIV-uninfected cohort is included in the proposed pooled analyses, as the primary objective of the HIV-infected cohort was evaluation of safety and immunogenicity (rather than efficacy) of influenza vaccine. Women were enrolled and vaccinated at ≥28 weeks of gestation in Mali, at 17–34 weeks of gestation in Nepal, and at ≥20 to <36 weeks of gestation in South Africa. Study subjects were followed from enrollment through delivery and approximately 6 months of infant age at all three sites (South Africa defined the follow up period as 24 weeks postpartum; whereas the other two sites defined it as 6 months). Details of differences in the eligibility criteria are described in Table 2. Moreover, all three trials individually randomized the enrolled women with a 1:1 randomization ratio using block randomization

Table 1
Comparison of maternal mortality ratios and infant mortality rates across Mali, Nepal, and South Africa.

	Trial site—morality rate and ratios		
	Mali	Nepal	South Africa
Maternal mortality ratio	540 per 100,000 live births (adjusted for underreporting and misclassification). [460 per 100,000 live births reported.] [33]	280 per 100,000 live births (adjusted for underreporting and misclassification). [170 per 100,000 live births reported.] [34]	400 per 100,000 live births (adjusted for underreporting and misclassification). [300 per 100,000 live births reported.] [35]
Infant mortality rate	50 per 1000 births country-wide estimate [36] 42 (24–70) per 1000 live births (2012) [37]	27 per 1000 births country-wide estimate [36] 24 (18–32) per 1000 live births (2012) [37]	19 per 1000 births country-wide estimate [36] 15 (9–26) per 1000 live births (2012) [37]

(the block sizes varied between the trials). In all three trials, the study subjects, investigators, and staff were blinded (except for statisticians, study pharmacists and, in case of Nepal and Mali, those responsible for vaccine administration). The vaccine brand, Vaxigrip (Sanofi Pasteur) inactivated influenza vaccine was the same for all three trials. In Nepal and Mali, both Northern and Southern hemisphere versions of the vaccine were used for year round vaccination; whereas, in South Africa, the Southern hemisphere version of the vaccine was used. In Nepal and South Africa, the vaccine contained the following antigens: A/California/7/2009 (H1N1)-like, A/Perth/16/2009 (H3N2)-like, B/Brisbane/60/2008-like during both years. In Mali, the vaccine had the same antigens as the other two sites from September 2011 through November 2012; however, from December 2012 through April 2013, the vaccine contained A/California/7/2009 (H1N1)-like, A/Victoria/361/2011 (H3N2)-like, B/Wisconsin/1/2010-like antigens.

The Mali trial used Menactra (Sanofi Pasteur), meningococcal polysaccharide diphtheria toxoid conjugate vaccine (groups A, C, Y, W-135) as the control vaccine. The Nepal and South Africa trials were placebo-controlled. In all trials, surveillance for maternal and infant outcomes was conducted through weekly home visits or telephonic contacts, and nasopharyngeal specimens were obtained from subjects meeting the clinical case definition (Table 2), as well as any unsolicited respiratory illness visit in South Africa. rRT-PCR was used at all three sites for laboratory confirmation of suspected cases (Table 3). South Africa utilized a 2 step rRT-PCR for universal detection of type A (subtypes H1 and H3) and B influenza viruses. Mali utilized a 1 step rRT-PCR to detect 2009 pandemic H1N1 and seasonal influenza viruses. Nepal utilized a 1 step rRT-PCR to detect seasonal influenza A (H1 and H3) and H1N1 subtypes.

In Mali, based on 90% power to detect 60% vaccine efficacy (reduction from 2.2% to 0.88% attack rate), 77 cases of laboratory-confirmed influenza in infants were required to meet primary objectives. While the trial originally planned for 5440 enrollments to achieve the 77 cases, this was not necessary as target laboratory-confirmed influenza was reached before then and enrollment was stopped with 4193 women vaccinated (one woman was inadvertently vaccinated twice). In Nepal, the total target enrollment was 3700 (i.e. 1850 in each of the 2 annual independently powered cohorts). The sample size was based on 90% power to detect 50% vaccine efficacy (reduction from 21.6 cases/100 person-years of PCR confirmed influenza illness in infants) in each of the two annual cohorts. In addition to influenza related outcomes, the Nepal trial was powered for birth outcomes including low birth weight, preterm birth, and small for gestational age. In South Africa, total target enrollment was 2108, based on 80% power to detect 50% vaccine efficacy (reduction from 5% attack rate of confirmed influenza illness in infants).

5. Outcome selection process

The outcomes for the pooled analysis were outlined by a working data group composed of investigators from the three trial sites, external experts, and BMGF personnel. Trial protocols, data dictionaries, and case report forms (where available) were reviewed to document similarities and differences among the three trials. Specific trial aspects considered for outcome selection included study design, inclusion and exclusion criteria, experimental and comparator vaccines, data collection time points, and study time periods. Outcome abstraction included only those outcomes related to influenza.

For data abstraction, protocols were reviewed for stated primary, secondary, tertiary, and other outcomes. If specific outcomes were not identified in trial protocols, case report forms and data dictionaries were consulted to determine whether data on those outcomes were collected. Outcome definitions were identified from trial protocols, where available. In some cases, we further specified outcome definitions using data dictionaries or case report forms. Where possible, specific case report forms used to capture outcomes were identified and, where applicable, absence of data on specific outcomes was noted. We then calculated available statistical power for each outcome in pooled analyses (see Supplement) and categorized potential outcomes according to available statistical power for within-site and pooled analyses, and the value added by conducting pooled analyses. The categories were as follows: Group A: Insufficient power for analysis within individual trials, but sufficient power in pooled analyses; Group B: Sufficient power for analysis within individual trials, but potential for added benefit of pooled analyses across by evaluating geographic variability and other site-specific factors, improving generalizability; Group C: Sufficient power for analysis within individual trials, limited potential for gain in efficiency through pooled analyses. The outcomes identified through this process, along with relevant categories, are presented in Supplement.

Through this process, 52 outcomes were identified for pooled analyses: 21 in category A, 17 in category B, and 14 in category C. We expect to group these outcomes into 11–13 analyses/manuscripts. The list of expected analyses is described in Box 1.

6. Power and pooled cohort size

We determined an assumed baseline rate or prevalence for each pooled outcome across the three trial sites based on three sources of information. First, we consulted trial protocols from the Mali, Nepal, and South Africa sites, as well as data available from the Mother's Gift trial in Bangladesh. Second, we searched PubMed for relevant literature with data on the pooled outcomes in Mali, Nepal, and South Africa, as well as summary estimates in nearby countries across Africa and Asia, or across developing countries. Third, we consulted reports from sources including the World Health Organization, March of Dimes, United Nations Children's Fund (UNICEF), United Nations Population Fund (UNFPA), and the Nepal Department of Health Services. A complete list of sources consulted is provided in the Supplement.

For binary and rate outcomes, we hypothesized that influenza vaccine would reduce the prevalence or rate of each outcome

Table 2
Comparison of study designs across maternal influenza immunization trials in Mali, Nepal, and South Africa.

	Trial site—study design and methods		
	Mali	Nepal	South Africa
Design	Randomized, controlled, observer-blind trial	Randomized, placebo controlled, community-based trial	Randomized, double-blind, placebo-controlled trial
Study population	Pregnant women receiving prenatal care at six referral centers and community health centers in Bamako. The community health centers are staffed by trained midwives while the referral centers have an obstetrician on staff	Women who are or who become pregnant in 9 Village Development Committees in Sarlahi District, Nepal. The healthcare centers within the Village Development Committees are staffed by traditional birth attendants	HIV-uninfected women accessing prenatal care at Chris Hani-Baragwanath Hospital or at one of four community-based antenatal clinics in Soweto region. The community-based clinics are staffed by midwives; in addition, women identified as having complicated or high-risk pregnancies would be assessed by medical doctors, including obstetricians at the hospital
Stated primary objectives	To compare the incidence of laboratory-confirmed influenza (LCI) among infants up to 6 months of age born to mothers immunized with trivalent influenza vaccine (TIV) during the 3rd trimester of pregnancy versus infants born to mothers who received meningococcal conjugate vaccine (MCV) during the 3rd trimester of pregnancy (intention-to-treat (ITT) comparison) To compare the incidence of LCI among infants up to 6 months of age born to mothers immunized with TIV during the 3rd trimester of pregnancy versus infants born to mothers who received MCV during the 3rd trimester of pregnancy, for infants born to women immunized ≥14 days prior to delivery	To compare the incidence of laboratory confirmed influenza illness episodes among newborn infants (through 6 months of age) born to women randomized to receive either influenza vaccine or control during pregnancy To compare the incidence of low birthweight (<2500 g) of newborn infants born to women randomized to receive either influenza vaccine or control during pregnancy To compare the incidence of influenza-like illness (ILI) episodes among pregnant women (through 6 months postpartum) in women randomized to receive either influenza vaccine or control during pregnancy	To determine the efficacy of TIV vaccination of pregnant women against laboratory-confirmed influenza illness, due to wild-type influenza strains which are homologous to vaccine-strains, in their infants up to 24 weeks of chronological age To evaluate the immunogenicity of TIV in pregnant women vaccinated between 20–36 weeks of gestational age
Enrollment	Third trimester of pregnancy (28 weeks or later)	17–34 weeks of gestation	≥20 to <36 weeks of gestation
Follow-up	Enrollment to infant 6 months of age	Enrollment to infant 6 months of age	Enrollment to infant 24 weeks of age
Major maternal eligibility criteria	In third trimester of pregnancy, intends to reside within study area until her newborn infant is 6 months of age	Lives within one of 9 selected VDCs; between 17 and 34 weeks gestation	≥18 to <39 years of age; gestational age 20–<36 weeks, HIV-1 uninfected
Major maternal exclusion criteria	History of severe influenza vaccine reaction, Guillain-Barre syndrome, egg allergy, chronic medical condition; known active infection with HIV, hepatitis B, or hepatitis C; complications with ongoing pregnancy (preterm labor, placental abruption, rupture of membranes, known major congenital anomaly, preeclampsia); acute illness or high temperature within 72 h of vaccination [temporary exclusion criterion]; receipt of any other vaccine excluding tetanus toxoid within 2 weeks (inactivated vaccines) or 4 weeks (live vaccines and meningococcal A conjugate vaccine); intends to travel out of study area in the 40 days after delivery; receipt of immunoglobulins or any blood products within 30 days of study vaccine; chronic usage of immunosuppressants or other immune-modifying agents within 90 days of study vaccine	Does not intend to deliver child within 9 VDCs in study area; already received current influenza vaccine; allergic to any component of vaccine; >34 weeks gestation. Also, excluded from primary analyses if delivers <2 weeks following receipt of study vaccine	Receipt of TIV (other than through the study) during current influenza season documented by medical history/record; receipt of any live licensed vaccine in last 28 days or inactivated licensed vaccine (except for TT) in last 14 days prior to study vaccine; receipt of non-licensed agent (e.g., vaccine, drug) in last 28 days before vaccination or plans to receive such before delivery; any significant acute illness and/or oral temperature (≥38 °C) in last 24 h prior to study entry [temporary exclusion criterion]; use of anti-cancer systemic chemotherapy or radiation in last 48 weeks before study enrollment or has immunosuppression as a result of underlying illness or treatment; long-term use of glucorticoids or high-dose inhaled steroids within 12 weeks of study entry; receipt of corticosteroids for preterm labor within 14 days before study entry; receipt of immunoglobulin or other blood products within 12 weeks before enrollment or is scheduled to receive such during pregnancy or for first 24 weeks after delivery; receipt of IL-2, IFN, GMCSF or other immune mediators within 12 weeks before enrollment; uncontrolled major psychiatric disorder; history of severe adverse reaction to previous TIV; pregnancy complications in current pregnancy (e.g., preterm labor, hypertension)

Table 2 (*Continued*)

	Trial site—study design and methods		
	Mali	Nepal	South Africa
Randomization	1:1 randomization at each health center, using blocks of size divisible by 2	1:1 randomization, blocked by VDC (Cohort 1) or by VDC and gestational age at vaccination [17–24 weeks, 25–34 weeks] because timing of vaccination not randomly/uniformly distributed (Cohort 2)	1:1 randomization, in blocks of 30 by enrollment site
Blinding	Observer-blinded (subjects and those involved in clinical surveillance for influenza and adverse reactions blinded), vaccination nurses not blinded	Only vaccinator will be un-blinded (will not be involved in assessment of reactogenicity or illness)	The statistician was responsible for generation of the randomization codes and therefore, was not blinded; however, the statistician was not involved in subject enrollment, case ascertainment or any other component of the study. All remaining staff in the data management team was blinded

Box 1: The list of expected pooled analyses.

- Estimating overall (pooled) efficacy of maternal influenza immunization against infant and maternal lab confirmed influenza. This analysis may also focus on determinants of variability in vaccine efficacy by site, season, and vaccine composition
- Impact of maternal influenza immunization on birth outcomes such as pre-term and small for gestational age births
- Immunogenicity of maternal TIV by site and antigen, dynamics of mother to infant antibody transfer. This analysis may also focus on determinants of variability in vaccine immunogenicity by site, vaccine composition, and maternal and infant characteristics
- Analysis of safety outcomes in mothers and infants—with a particular focus on endpoints too rare to be evaluated in individual trials (e.g., miscarriage, stillbirth)
- Impact of maternal TIV on neonatal mortality—all cause and, where possible, cause specific mortality
- Impact of maternal TIV on maternal mortality
- Infant growth by maternal vaccination status
- Indirect/"herd" effects of maternal TIV. Influenza-like illness and laboratory confirmed influenza among household contacts (Mali and Nepal only)
- Impact of maternal TIV on infant Pneumonia
- Impact of maternal TIV on (a) Medically Attended Acute Respiratory Illness (MAARI) among mothers and infants, and (b) Severe acute respiratory infection (mothers only). The working group might decide to recommend separate analyses for mothers and infants

by 10–50%. Therefore, it was assumed that the difference to be detected was an Odds Ratio of 0.5 to 0.9 (by 0.05 increments) for binary outcomes and an Incidence Rate Ratio of 0.5 to 0.9 (by 0.05 increments) for rate outcomes. For continuous outcomes and proportions, we assumed a difference between means or proportions to be detected between the influenza vaccine and control groups based on available data on baseline prevalence. NCSS Power Analysis & Sample Size (PASS) software versions 11 and 12 (Kaysville, UT) was used for power calculations.

For pooled outcomes that will be analyzed across all three trial sites, a sample size of 8500 to 11,500 (by 500 increments) was assumed. This sample size is based on the sum of the total target enrollments across the three trials (5440 in Mali; 3700 in Nepal; 2116 in South Africa), accounting for up to approximately 25% loss to follow up. For certain outcomes, data are only available from two of the three trial sites; therefore, the assumed sample size was adjusted accordingly. In addition, within-site power for certain outcomes was computed, assuming a sample size of 1500 to 4000 (by 500 increments) based on the sizes of the three trials.

7. Analytical approaches for pooled analyses

Our overall goal is to conduct methodologically appropriate analyses to generate results that could be communicated to a range of stakeholders including researchers, policy makers, clinicians, and public health practitioners. This pooled analyses is designed to be conducted after the end of enrollment and follow up at each site. Our analytical approach was informed by this goal. We anticipate our primary analyses to use fixed effects model with evaluation of an interaction term for site. If significant interaction is found, then this term will be included in the final model. In order to account for heterogeneity among the three sites, we may use a cluster term for trial site to generate robust confidence intervals. Where appropriate, we will conduct sensitivity analyses using random effects model, with random intercept for site. This will treat variability across sites as a nuisance parameter, and will give a single estimate for the effect of vaccine on each outcome. In some cases, we will consider evaluating random slope for the effect of vaccine by site.

8. Expectations from and interpretations of trial results

While the results from the BMGF-sponsored maternal immunization trials are likely to strengthen the evidence base regarding the impact of influenza immunization in pregnancy, expectations from these results should be realistic. For example, evidence from previous influenza vaccine studies – conducted in general, non-pregnant populations – suggests substantial geographic and year-to-year variability in influenza incidence and severity, as well as vaccine efficacy/effectiveness. In this section, we discuss a few sources of heterogeneity and the role of underlying effect modifiers that could impact findings (and their interpretations) from the maternal influenza immunization trials.

8.1. Variations in influenza epidemiology

There is known seasonal variation in influenza disease patterns. In a systematic review of seasonal influenza epidemiology in sub-Saharan Africa [16], studies of seasonality reported over at least 12 consecutive months showed strong seasonality in Zambia, Madagascar, and South Africa (southern Africa) and weak seasonality in Senegal (closer to the equator). Another review of influenza surveillance data from 85 countries between 1983 and 2008 compared the timing of seasonal epidemic influenza activity in tropical, subtropical, and temperate regions [17]. The majority of countries had one annual influenza epidemic (often comprising of multiple viruses) rather than having multiple epidemics per year: 85% (40/47) of temperate countries (i.e. countries with latitude >30°), 100% (6/6) of subtropical countries (i.e. latitude 23.6–29°), and 56% (18/32) of tropical countries (i.e. latitude ≤23.5°). Countries with year-round influenza activity ($n = 15$) were more likely to be located in the tropics, specifically in Southeast Asia. Across all countries included in the review, the mean duration of influenza epidemics was 4

Table 3
Comparison of surveillance and diagnostic assays across maternal influenza immunization trials in Mali, Nepal, and South Africa.

	Trial site—surveillance and diagnostic assays		
	Mali	Nepal	South Africa
Maternal study vaccine	Vaxigrip (Sanofi Pasteur), inactivated trivalent influenza vaccine. Southern hemisphere versions. From 09/2011 to 11/2012, A/California/7/2009 (H1N1)-like, A/Perth/16/2009 (H3N2)-like, B/Brisbane/60/2008-like. From 12/2012 to 04/2013, A/California/7/2009 (H1N1)-like, A/Victoria/361/2011 (H3N2)-like, B/Wisconsin/1/2010-like	Vaxigrip (Sanofi Pasteur), inactivated trivalent influenza vaccine. Southern and Northern hemisphere versions, containing A/California/7/2009 (H1N1)-like, A/Perth/16/2009 (H3N2)-like, B/Brisbane/60/2008	Vaxigrip (Sanofi Pasteur), inactivated trivalent influenza vaccine. Southern hemisphere version, containing A/California/7/2009 (H1N1)-like, A/Perth/16/2009 (H3N2)-like, B/Brisbane/60/2008
Control	Menactra (Sanofi Pasteur), meningococcal polysaccharide diphtheria toxoid conjugate vaccine (groups A, C, Y, W-135)	Placebo (saline injection)	Placebo (saline injection)
Timing of vaccine administration	Third trimester of pregnancy (28 weeks or later)	17–34 weeks gestation	≥20 to <36 weeks gestation
Maternal influenza surveillance	Weekly visits	Weekly home visits	Weekly visits
Infant influenza surveillance	Weekly visits	Weekly home visits	Weekly visits
Clinical definition of influenza in mother	Laboratory-confirmed influenza: positive nasopharyngeal swab specimen for influenza (PCR testing) Influenza-like illness: Case of febrile influenza-like illness in mothers according to study case definition as follows: If observed by the examining physician or part of clinical history: −Onset of fever (oral temperature ≥ 38 °C) < 7 days duration AND −Cough or sore throat AND −Absence of other diagnoses OR −Onset of feverish feeling <7 days duration AND −Cough or sore throat or chest pain on breathing in AND −Absence of other diagnoses OR −Sudden onset of fever over 38 °C or perception of fever and self-administration of antipyretic in the previous 8 h AND −Cough or sore throat AND −Shortness of breath or difficulty breathing −Patient may or may not be hospitalized. NB: This is the definition of severe acute respiratory infection	Laboratory-confirmed influenza: An episode of respiratory illness (reported or measured fever (>38 °C) plus one or more of the following: cough, sore throat, runny nose, nasal congestion, or myalgia) plus a positive laboratory test for influenza from nasal swab(s) Episodes must be separated by 7 or more days Influenza-like illness: CDC definition of ILI, requires reported or measured fever (>38 °C) plus either cough or sore throat on one or more days Episodes of ILI must be separated by 7 or more days	Laboratory-confirmed influenza: Positive rRTPCR test for influenza virus Case of laboratory-confirmed influenza in mother defined as: Adult participant with laboratory-confirmed influenza (PCR from NP/OP swab positive for influenza A or B) Influenza-like illness: Case of influenza-like illness in mother according to study case definition as follows: −Fever (≥38 °C on oral measurements) or chills/rigor or feeling feverish in past 7 days AND −Cough/sore throat/pharyngitis OR −Muscle, joint, or headache OR −Feeling short of breath, had difficulty breathing or chest pain while breathing AND −Absence of other diagnoses

Clinical definition of influenza in infant	Laboratory-confirmed influenza: Positive nasopharyngeal swab specimen for influenza (PCR testing) Influenza-like illness: Case of febrile influenza-like illness in infants according to study case definitions as follows 0–5-month-olds: Either of the 2 following conditions reported by caretaker or observed by clinician: (1) Fever w/o apparent source, documented by clinician's measurement to be an axillary temperature > = 38 °C or maternal perception of fever and administration of antipyretic in previous 8 h (no source means there is no apparent cause for the fever such as soft tissue infection, although generalized symptoms such as irritability, loss of appetite, and/or lethargy may be present; OR (2) Fever (as defined below)* plus acute respiratory infection. Acute respiratory infection is defined as ANY of the following on the same or consecutive days: runny nose, nasal congestion, cough, difficulty breathing, pus draining from ear or wheezing; PLUS >7 days after last reported fever 6–59-month olds: Either of the following conditions reported by caretaker or observed by clinician: (1) Fever w/o apparent source, documented by a clinician's measurement to be an axillary temperature ≥38 °C or maternal perception of fever and administration of antipyretic in previous 8 h (No source means that there is no apparent cause for the fever such as soft tissue infection, although generalized symptoms such as irritability, loss of appetite, and/or lethargy may be present; OR (2) Fever (as defined below)* plus acute respiratory infection (acute respiratory infection defined as ANY of the following on the same or consecutive days: runny nose, nasal congestion, cough, difficulty breathing, wheezing, sore throat, headache, earache, muscle ache); PLUS >7 days after last reported fever Definition of fever: Any of the following – (1) Mother's perception that child had a fever during the previous 24 h; (2) Mother measured the child's temperature as >38 °C during the previous 24 h; (3) Clinician or study staff measure the child's temperature to be >38 °C; (4) Maternal perception of fever and administration of antipyretic in previous 8 h	Laboratory-confirmed influenza: An episode of respiratory illness (one or more of the following: reported or measured fever (>38 °C), cough, runny nose, wheeze, difficult or rapid breathing, draining ear) plus a positive laboratory test for influenza from nasal swab Episodes must be separated by 7 or more days Influenza-like illness: Modified CDC definition of influenza-like illness, including reported or measured fever (>38 °C) plus cough, or runny nose, or draining ear, or nasal congestion occurring on one or more days Episodes of ILI must be separated by 7 or more days	Laboratory-confirmed influenza: Positive rRTPCR test for influenza virus Case of laboratory-confirmed influenza defined according to study case definition as follows: Infant with laboratory-confirmed influenza (PCR from nasal sample positive for influenza A or B) and at least one of the following: fever, cough, runny nose, wheezing, difficulty breathing, tachypnea Influenza-like illness: Case defined according to study protocol case definition, as follows Fever (≥37.8 °C axillary temperature) of acute onset (<7 days) without an apparent source, as documented by parent/caregiver/study staff (no source means that there is no apparent cause for the fever, such as soft tissue infection, although generalized symptoms such as irritability, loss of appetite, and/or lethargy may be present); OR Fever (documented as ≥37.8 °C and/or mother's perception that infant is feverish/hot) plus at least one sign/symptom of acute respiratory infection within the past 72 h; OR At least 2 signs/symptoms of ARTI within the past 72 h: –Tachypnea (RR≥60 breaths/min in infant 0-2 months of age; RR≥50 breaths/min in infant 2-12 months of age); –Difficulty breathing (reported by mother: noisy/interrupted/irregular/or fast) –Coughing –Wheezing –Runny or congested nose –Cyanosis/O_2 saturation <90% (if available) –Chest wall indrawing –Grunting on expiration –Pus draining from ear

Table 3 (*Continued*)

	Trial site—surveillance and diagnostic assays		
	Mali	Nepal	South Africa
Maternal nasopharyngeal swab collection & testing	If influenza-like symptoms present at weekly visit Possible PCR test results are influenza A/human H1, A/human H3, B, pandemic H1N1 (swine influenza), H5a or H5b (avian influenza). 25% sample of specimens that are positive for influenza A viruses of swine H1N1, "human" H1N1 or H3N2 type, or influenza B viruses, will be antigenically characterized	If influenza-like symptoms present at weekly visit Specimens tested using PCR for influenza A. Specimens testing positive for influenza A will be subtyped for seasonal influenza A H1 and H3 subtypes and the novel H1N1 (swine origin) subtype using real time one-step RT-PCR assays	If influenza-like symptoms present at weekly visit If presenting with unsolicited respiratory illness, irrespective of fulfilling ILI criteria Specimens tested using PCR. Specimens testing positive for influenza A will be subtyped as H1 or H3 subtypes. All influenza-positive samples also sequenced to determine "vaccine match" to vaccine strains for H1 and H3
Infant nasopharyngeal swab collection	If influenza-like symptoms present at weekly visit Possible PCR test results are influenza A/human H1, A/human H3, B, pandemic H1N1 (swine influenza), H5a or H5b (avian influenza). 25% sample of specimens that are positive for influenza A viruses of swine H1N1, "human" H1N1 or H3N2 type, or influenza B viruses, will be antigenically characterized Note: In Mali, nasal and throat swabs were collected	If influenza-like symptoms present at weekly visit Specimens tested using PCR. Specimens testing positive for influenza A will be subtyped for seasonal influenza A H1 and H3 subtypes and the novel H1N1 (swine origin) subtype using real time one-step RT-PCR assays	If influenza-like symptoms present at weekly visit If presenting with unsolicited respiratory illness, irrespective of fulfilling ILI criteria Specimens tested using PCR. Specimens testing positive for influenza A will be subtyped as H1 or H3 subtypes. All influenza-positive samples also sequenced to determine "vaccine match" to vaccine strains for H1 and H3
Maternal serum collection	Vaccine delivery, 1 month post-vaccination, delivery, 3 months post-delivery, 6 months post-delivery	Enrollment, delivery	Enrollment, 1 month post-vaccination (28-35 days), delivery (within 1 week of birth) and at 24 weeks post-partum[only in immunogenicity sub-cohort]
Infant serum collection	Delivery (cord blood if delivered at study health center; if not, peripheral blood collected before day 7 after birth), 3 months of age, 6 months of age	Delivery (cord blood)	Delivery (within 1 week of birth), 8 weeks of age, 16 weeks of age, and 24 weeks of age [only in immunogenicity sub-cohort]
Infant specimen collection for other pathogens	Nasal and throat swabs taken when infants meet criteria for influenza-like illness, and from 1/3 of healthy infants at 3 and 6 months of age	Viral and pertussis assays performed on nasal swabs	NP swabs for *S. pneumoniae* and *S. aureus* at 8 weeks of age, 16 weeks of age, 24 weeks of age, and during weekly visits if respiratory illness
Maternal specimen collection for other pathogens	Nasal and throat swabs taken when mothers meet criteria for influenza-like illness	Viral and pertussis assays performed on nasal swabs	NP and OP swabs for *S. pneumoniae* colonization at weekly visits if suspected of pneumonia
Planned sample size	Total target enrollment was 5440, based on 90% power to detect 60% vaccine efficacy (reduction from 2.2% to 0.88% attack rate), with 5% type 1 error rate, accounting for 20% loss to follow-up due to death, withdrawal, and exclusion of mothers receiving vaccine <14 days prior to delivery from primary analysis Required 77 cases of laboratory-confirmed influenza in infants to meet primary objectives. While originally planned for 5440, this was not necessary as target laboratory-confirmed influenza was reached before then	Total target enrollment was 1850 in each of 2 cohorts, based on 90% power to detect 50% vaccine efficacy (reduction from 21.6 cases/100 person-years), with 1.7% type 1 error rate, accounting for 6 months follow-up per infant, stillbirth/miscarriage, and loss to follow up	Total target enrollment was 2116, based on 80% power to detect 50% vaccine efficacy (reduction from 5% attack rate), with 5% type 1 error rate, accounting for 10% loss to follow up Target number of cases of laboratory-confirmed influenza illnesses due to wild-type influenza virus (homologous to vaccine strain) was 27, to detect 70% reduction in laboratory-confirmed influenza illness in infants, based on 80% power and 80% of infants being born during the influenza season.
Assumed baseline incidence of laboratory-confirmed influenza among infants in study area	2.2% attack rate for laboratory-confirmed influenza in infants (counting only first cases of influenza)	21.6 cases of proven influenza illness/100 person-years among infants 0–6 months of age	5% attack rate in infants
Dates of enrollment	9/12/2011 to 4/19/2013	Cohort 1: mid-April 2011 to mid-April 2012 Cohort 2: mid-April 2012 to mid-April 2013	Started 3 March 2011 and ended 4 Aug 2012 for 1st cohort and 2nd cohort enrolled from 6 March 2012 to 2 July 2012

Table 4
Comparison of Outcomes across Maternal Influenza Immunization Trials in Mali, Nepal, and South Africa.

	Trial site—outcomes		
	Mali	Nepal	South Africa
Primary	Influenza infection: Laboratory-confirmed influenza (infant)	Birth outcomes: Low birthweight Influenza infection Influenza-like illness (mother) Laboratory-confirmed influenza (infants)	Immunogenicity: Seroprotection Influenza infection: Laboratory-confirmed influenza (infant)
Secondary	Birth outcomes: Congenital malformations Low birthweight Other birth complications Preterm birth Hospitalization: Hospitalizations for laboratory-confirmed influenza (infant) Immunogenicity: Geometric mean titer for infant Maternal/fetal HAI titer ratio (infant) Cell-mediated immune response to each influenza strain (mother) Geometric mean titer (mother) Regulatory B cell subpopulation (mother) Regulatory T cell subpopulation (mother) T-cell activation (mother) Seroprotection, by absolute titer for infant Influenza antibody in breast milk Influenza infection: Influenza-like illness in infants Influenza-like illness (mother) Laboratory-confirmed influenza (mother) Laboratory-confirmed influenza illness due to all wild-type strains, irrespective of match to vaccine strain (mother) Laboratory-confirmed influenza illness due to wild-type influenza strains, irrespective of match to vaccine strain (infant) Laboratory-confirmed influenza illness due to wild-type influenza strains that are heterologous to vaccine strains (infant) Laboratory-confirmed influenza illness due to wild-type influenza strains that are heterologous to vaccine strains (mother) Pneumonia: S. pneumoniae carriage fever (infants) Other pathogens: In infants In mothers Pregnancy complications: Preeclampsia Stillbirth Gestational hypertension Safety: Any SAE (mother) Any SAE (mother) Fatigue (mother) Fever (mother) Headache (mother) Malaise (mother) Myalgia (mother) Nausea (mother) Rash (mother) Presence of redness at injection site (mother) Swelling at injection site (mother) Tenderness (mother) Pain at injection site (mother) Meningitis: Geometric mean titer (mother) Fatigue Fever Headache Myalgia Pain at injection site Redness at injection site Swelling at injection site Serious adverse event	Birth outcomes: Gestational age Preterm birth Small for gestational age Hospitalization: Hospitalizations for laboratory-confirmed influenza (infant) Hospitalizations (infant) Hospitalizations (mother) Influenza infection: Influenza-like illness in household contacts Influenza-like illness in infants Laboratory-confirmed influenza in household contacts Laboratory-confirmed influenza in mothers Other pathogens: Infant Mother Pregnancy complications: Miscarriage Stillbirth Clinic visits: Clinic visits for influenza-like illness (infant) Clinic visits for influenza-like illness (mother) Hepatitis: Acute viral hepatitis Anti-HEV IgG prevalence Biochemical correlates of clinical HEV disease, among women who seroconvert Disease to infection ratio for HEV, among women who seroconvert HEV genotypes present in incident cases HEV infection (anti-HEV seroconversion) Risk factors for HEV seroconversion Infant outcomes: Infant growth Infant outcomes: Neonatal mortality Maternal outcomes: Maternal mortality	Birth outcomes: Low birthweight Preterm birth Other birth complications Hospitalization: Hospitalizations for laboratory-confirmed influenza, in infants Hospitalizations, in infants Immunogenicity: Geometric mean titer (infant) Maternal/fetal HAI titer ratio (infant) Cell-mediated immune response to each influenza strain (mother) Geometric mean titer (mother) Regulatory B cell subpopulation (mother) Regulatory T cell subpopulation (mother) T-cell activation (mother) Seroprotection, by absolute titer (infant) Influenza antibody in breast milk Influenza infection: Influenza-like illness in infants Influenza-like illness in mothers Laboratory-confirmed influenza illness due to all wild-type strains, irrespective of match to vaccine strain, in mothers Laboratory-confirmed influenza illness due to wild-type influenza strains, irrespective of match to vaccine strain, in infants Laboratory-confirmed influenza illness due to wild-type influenza strains that are heterologous to vaccine strains, in infants Laboratory-confirmed influenza illness due to wild-type influenza strains that are heterologous to vaccine strains, in mothers Laboratory-confirmed influenza in mothers Pneumonia: S. pneumoniae carriage (infant) Other pathogens: Infant Mother Safety: Any SAE in infant Any SAE in mother Fever (mother) Headache (mother) Malaise (mother) Myalgia (mother) Nausea (mother) Rash (mother) Presence of redness at injection site (mother) Swelling at injection site (mother) Tenderness (mother)

Table 4 (*Continued*)

	Trial site—outcomes		
	Mali	Nepal	South Africa
Tertiary	Birth outcomes: Birth weight Cost: Cost of influenza-like illness in infants up to 6 months Cost of influenza-like illness in pregnant women up to 6 months post-partum Cost of laboratory-confirmed influenza in infants up to 6 months Cost of laboratory-confirmed influenza in pregnant women up to 6 months post-partum Immunogenicity: Geometric mean titer (infants) Maternal/fetal HAI titer ratio (infants) Influenza infection: Asymptomatic infection with influenza virus in healthy infants at 3 and 6 months of age Laboratory-confirmed influenza in household contacts Influenza virus circulation: Influenza virus types circulating in the study population Meningitis: Geometric mean titer (infants) Meningococcal disease in infants Serogroup-specific serum bactericidal antibodies (A, C, Y, and W-135) in pregnant women Other pathogens (infant) Severe acute respiratory infection (mother)		

months, and peak influenza activity was modestly associated with lower temperature.

There are several potential reasons for heterogeneity in influenza epidemiology. Tamerius et al. reviewed evidence for the following potential seasonal mechanisms explaining seasonal patterns of influenza disease activity: seasonal variations in (1) host contact rate, (2) virus survival, and (3) host immunity [18].

It has been hypothesized that frequency of contacts between infected and susceptible hosts is seasonally patterned (e.g., increased frequency of contact due to indoor crowding in colder months) [19]. Contact rates may be influenced by school schedules, temperature, travel and workflow patterns, and precipitation (e.g., colder weather in temperate regions, rainy weather in tropical regions). However, some studies have found contradictory results, such as winter influenza epidemics in desert regions of the U.S. where there is likely higher crowding during hot summer months. Therefore, the authors concluded that there is a lack of strong empirical data to support this contact hypothesis, and that this mechanism likely operates in combination with other causes of seasonal variation in influenza [18].

Moreover, seasonal variation in influenza disease patterns could be due to differences in virus survival, in response to temperature, relative humidity, absolute humidity, and exposure to ultraviolet radiation (e.g., sun exposure) [18]. Lower absolute humidity tends to be associated with higher virus survival among guinea pigs; however, the occurrence of tropical influenza epidemics during the rainy season cannot be explained by this effect [18]. Higher virus survival may be associated with less exposure to ultraviolet radiation; this hypothesis could explain influenza epidemics in both temperate regions (winter) and tropical regions (rainy season) [20].

There may also be seasonal variations in hosts' general immunity to influenza, which make them less likely to develop influenza-like symptoms during non-epidemic months [18]. This may be influenced by temperature and humidity, exposure to sunlight and vitamin D levels, nutrient availability, changes in airborne particulate matter and pollutants, higher energy required for thermoregulation during winter or rainy seasons, and interaction between influenza virus and other pathogens [18].

8.2. Variability in vaccine efficacy/effectiveness

Osterholm et al. conducted a systematic review and meta-analysis of randomized controlled trials and observational studies of inactivated influenza vaccine [21]. In this analysis, including 8 randomized controlled trials of trivalent inactivated vaccine (TIV) conducted in 31,892 adults aged 18–64 years over 9 influenza seasons, 5 trials found a significant protective effect of TIV. The random-effects pooled vaccine efficacy was 59% (95% CI: 51–67). The vaccine efficacy among healthy adults ranged from 16% to 75% with substantial variability from season to season and between studies [21]. Moreover, in one randomized controlled trial of TIV in children aged 6–24 months conducted over two influenza seasons, vaccine efficacy was 66% in the first season (1999–2000) and −7% in the second season (2000–2001). Both seasons had a good match between vaccine and circulating strains. Nine observational studies of seasonal influenza vaccine effectiveness comprising of 17 seasonal or separate cohort analyses were eligible for inclusion in this review. Among the 17 analyses, 6 (35%) reported a significant protective effect of seasonal influenza vaccine against medically attended, laboratory-confirmed influenza with substantial variability between seasons.

8.3. Effects of vaccine match

Antigenic mismatch between vaccine and circulating strain affects vaccine effectiveness. For example, among healthy 18–64 year olds in the U.S., vaccine effectiveness for the poor match 1997–1998 influenza season was 50% (not significantly protective). In comparison, vaccine effectiveness for the good match 1998–1999 influenza season was 80% (significantly protective) [22]. Among children 6–23 months old and adults ≥65 years old in Wisconsin, vaccine effectiveness varied across three influenza seasons with differing antigenic match. In the 2004–2005 and 2005–2006 influenza seasons, when match was poor, VE was −10% (95% CI: −36–40) and 21% (95% CI: −52–59), respectively. In the 2006–2007 influenza season, when match was good, VE was 52% (95% CI: 22–70) [23]. Moreover, in a systematic review and meta-analysis

by DiazGranados, Denis, and Plotkin [24]; randomized or quasi-randomized controlled trials were eligible for inclusion. Overall vaccine efficacy of inactivated vaccines (any type/subtype) was 56% (95% CI: 48–63) for any strain/season, 66% (95% CI: 49–77) for matched strains, 55% (95% CI: 42–65) for not matched strains, 54% (95% CI: 42–64) for good match season; and 49% (95% CI: 28–64) for poor match season.

8.4. Underlying effect modifiers

An additional aspect of maternal influenza immunization is the potential for a protective effect of influenza vaccine in pregnancy on neonatal outcomes such as preterm, small for gestational age births, and low birth weight [3,14]. Since these birth outcomes have a multifaceted etiology, the variability in underlying rates of covariates such as maternal nutritional status, obesity, and baseline maternal antibody titer due to previous infection and/or vaccination can influence the efficacy of maternal influenza vaccination against birth outcomes.

Moreover, trans-placental transfer of maternal antibodies is an important mechanism for infant protection against influenza. Mother-to-infant IgG transfer has been shown to be influenced by maternal co-infection. For example, among women who received antenatal tetanus toxoid, malaria infection during pregnancy has been associated with reduced infant antibody levels [25,26]. Tetanus antibody titers were 48% lower among neonates of women with documented placental malaria when compared with neonates born without evidence of placental malaria infection [25]. However, findings of an impact of malaria infection on antibody transfer have not been consistent. For example, studies in Malawi and The Gambia found no association between placental malaria and tetanus antibody levels in the newborn [27]. It could be that the differing results for the impact of malaria infection on antibody transfer relate to variations in prevalence of severe malaria.

Similarly, maternal HIV infection has been observed to have variable effect on efficiency of transplacental IgG transfer, differing for antibody to different epitopes [28]. However, in general HIV-infected mothers had lower antibody levels, albeit similar efficiency of transfer in terms of geometric mean ratios (GMRs) for trans-placental transfer [28–30]. There are few studies on the impact of HIV infection on transfer of maternal influenza antibody. A recent trial of 2009 pH1N1 influenza immunization during pregnancy among HIV-infected pregnant women showed 65% with sero-protective HAI titers ($\geq$1:40) among infants at birth, which waned to 26% at 3 months and 12% at 6 months [31].

While there are data on the impact of malaria and HIV on maternal antibody transfer, these data do not include influenza antibody transfer. Given the possibility of maternal co-infection (e.g., with HIV and/or malaria) modifying transfer of influenza antibodies to the infant, there is a need to characterize the influence of maternal co-infection on influenza antibody transfer, an issue which will be addressed in the BMGF South African study involving an HIV-infected cohort. Moreover, there is a need for data on maternal efficacy of maternal influenza vaccine from locations with high HIV and/or malaria prevalence.

9. Beyond the pooled analyses

In 2013, GAVI Alliance explored the possibility of funding seasonal influenza vaccination for pregnant women as part of their Vaccine Investment Strategy (VIS) development process. Influenza vaccine was not recommended for this round of investments—primarily due to the "high degree of uncertainty around the estimates of influenza vaccination impact" as there has only been a single randomized controlled trial of maternal immunization in a low-income country [32]. However, this uncertainty is likely to be resolved after the results from the three BMGF funded trials become available. The VIS recommended that "the [GAVI] Board may want to consider preparatory activities to facilitate re-evaluation of influenza vaccine support in the next VIS process. Such preparatory activities could focus on acquiring additional data on implementation feasibility and addressing questions relating to the logistics of seasonal vaccine supply, surveillance and strain matching, and optimal delivery strategies for pregnant women." [32]. Therefore, in order to facilitate decision making regarding broad adoption of maternal influenza immunization in developing countries, data beyond the three trials will be needed. We will also encourage documenting lessons learned and best practices from low and middle income countries with influenza vaccination policies.

Specific examples of additional data gaps include logistics for integrating maternal influenza immunization into antenatal care delivery in low and middle income countries. While low and middle income countries have substantial experience in delivering tetanus toxoid (or tetanus and diphtheria toxoid) in pregnancy, moving from a single vaccine to a multiple dose schedule will require consideration and assessment of supply chain logistics, barriers to integration into the staff workflow, and understanding of vaccine acceptance among pregnant women and their health care providers. Moreover, selection of a vaccine product with a strain composition relevant to countries with perennial influenza circulation, and deciding whether some countries need both Northern and Southern vaccine at different times of the year, will require ongoing acquisition and synthesis of data on influenza circulation in low and middle income countries.

There is need for additional data to determine the optimal timing of influenza vaccination during pregnancy. For example, depending on the effects of immunization on various outcomes, it may be more important to protect the woman for longer during the pregnancy than giving it later in pregnancy. However, the challenge is that many women in developing countries seek care late in pregnancy and maternal outcomes of influenza are worse later in pregnancy.

Moreover, studies so far – including the three BMGF supported trials – have primarily focused on mild to moderately severe disease. Evaluating the impact on severe outcomes, such as pneumonia and hospitalization, would yield information on the full benefit of maternal influenza immunization. Furthermore, there have been a few cost effectiveness analyses of maternal influenza immunization. For example, a cost effective analysis from Mali found that the impact on birth outcomes such as birth weight would be the most important driver of cost effectiveness (unpublished data). However, as the data from the three trials (and additional observational data) become available, the cost effectiveness models may need to be upgraded.

10. Conclusions

Maternal influenza immunization is a promising strategy to reduce morbidity and mortality associated with influenza among pregnant women and young infants. The results from the three BMGF-sponsored trials are likely to substantially increase the evidence base for the impact of maternal influenza immunization.

BMGF Supported Maternal Influenza Immunization Trials Investigators Group

Ordered by site and alphabetically.

Mali: William Blackwelder, PhD; Joseph Bresee, MD; Flanon Coulibaly, MD; Boubacar Diallo; Fatoumata Diallo, MD; Wilbur

Chen, MD, MS; Moussa Doumbia, MD; Fadima Cheick Haidara, MD; Adama Mamby Keita, MD, Alexander Klimov, PhD; Mamoudou Kodio, PharmD; Karen Kotloff, MD; Myron M. Levine, MD, DTPH; Uma Onwuchekwa; Sandra Panchalingam, PhD; Marcela Pasetti, PhD; Doh Sanogo, MD; Samba Sow, MD MS; Milagritos Tapia, MD; Boubou Tamboura, PharmD; Ibrahim Teguete, MD; Sharon Tennant, PhD, Awa Traore, PharmD; and John Treanor, MD.

Nepal: Janet A. Englund, MD; Joanne Katz, ScD; Subarna K. Khatry, MD; Jane Kuypers, PhD; Alain B. Labrique, PhD; Steven C. LeClerq, MPH; Luke C. Mullany, PhD; Laxman Shrestha, MD; Mark C. Steinhoff, MD; and James M. Tielsch, PhD.

South Africa: Peter V. Adrian, PhD; Clare L. Cutland, MD; Andrea Hugo, MD; Stephanie Jones, MD; Locadiah Kuwanda, MSc; Keith P. Klugman, MD, PhD; Shabir A. Madhi, MD, PhD; Kathleen M. Neuzil, MD; Nadia van Niekerk, BTech; Marta C. Nunes, PhD; Justin R. Ortiz, MD; Eric A.F. Simões, MD; Florette Treurnicht, PhD; Marietjie Venter, PhD; Avy Violari, MD; and Adriana Weinberg, MD.

Conflict of interest statement

None.

Acknowledgements

The three trials in Mali, South Africa, and Nepal as well as this pooled analyses are funded by the Bill and Melinda Gates Foundation (24090 and 26363).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2015.05.077

References

[1] McNeil SA, Dodds LA, Fell DB, et al. Effect of respiratory hospitalization during pregnancy on infant outcomes. Am J Obstet Gynecol 2011;204(6 (Suppl 1)):S54–7.
[2] Acs N, Banhidy F, Puho E, Czeizel AE. Maternal influenza during pregnancy and risk of congenital abnormalities in offspring. Birth Defects Res A Clin Mol Teratol 2005;73(12):989–96.
[3] Omer SB, Goodman D, Steinhoff MC, et al. Maternal influenza immunization and reduced likelihood of prematurity and small for gestational age births: a retrospective cohort study. PLoS Med 2011;8(5):e1000441.
[4] Pierce M, Kurinczuk JJ, Spark P, Brocklehurst P, Knight M. Perinatal outcomes after maternal 2009/H1N1 infection: national cohort study. BMJ 2011;342:d3214.
[5] Mendez-Figueroa H, Raker C, Anderson BL. Neonatal characteristics and outcomes of pregnancies complicated by influenza infection during the 2009 pandemic. Am J Obstet Gynecol 2011;204(6 (Suppl 1)):S58–63.
[6] Rasmussen SA, Jamieson DJ, Uyeki TM. Effects of influenza on pregnant women and infants. Am J Obstet Gynecol 2012;207(3 (Suppl)):S3–8.
[7] Tamma PD, Steinhoff MC, Omer SB. Influenza infection and vaccination in pregnant women. Expert Rev Respir Med 2010;4(3):321–8.
[8] Goodnight WH, Soper DE. Pneumonia in pregnancy. Crit Care Med 2005;33(10 (Suppl)):S390–7.
[9] Gabbe S. Obstetrics: normal and problem pregnancies. 5th ed. Philadelphia, PA: Churchill Livingstone/Elsevier; 2007.
[10] Adegbola R, Nesin M, Wairagkar N. Immunogenicity and efficacy of influenza immunization during pregnancy: recent and ongoing studies. Am J Obstet Gynecol 2012;207(3 (Suppl)):S28–32.
[11] Madhi SA, Cutland CL, Kuwanda L, et al. Influenza vaccination of pregnant women and protection of their infants. N Engl J Med 2014;371(10):918–31.
[12] S.W. Group. Background paper on influenza vaccines and immunization. World Health Organization; 2012.
[13] Zaman K, Roy E, Arifeen SE, et al. Effectiveness of maternal influenza immunization in mothers and infants. N Engl J Med 2008;359(15):1555–64.
[14] Steinhoff MC, Omer SB, Roy E, et al. Neonatal outcomes after influenza immunization during pregnancy: a randomized controlled trial. CMAJ 2012;184(6):645–53.
[15] Hambidge SJ, Newcomer SR, Narwaney KJ, et al. Timely versus delayed early childhood vaccination and seizures. Pediatrics 2014:e1492–9.
[16] Gessner BD, Shindo N, Briand S. Seasonal influenza epidemiology in sub-Saharan Africa: a systematic review. Lancet Infect Dis 2011;11(3):223–35.
[17] Azziz Baumgartner E, Dao CN, Nasreen S, et al. Seasonality, timing, and climate drivers of influenza activity worldwide. J Infect Dis 2012;206(6):838–46.
[18] Tamerius J, Nelson MI, Zhou SZ, Viboud C, Miller MA, Alonso WJ. Global influenza seasonality: reconciling patterns across temperate and tropical regions. Environ Health Perspect 2011;119(4):439–45.
[19] Lofgren E, Fefferman NH, Naumov YN, Gorski J, Naumova EN. Influenza seasonality: underlying causes and modeling theories. J Virol 2007;81(11):5429–36.
[20] Sagripanti JL, Lytle CD. Inactivation of influenza virus by solar radiation. Photochem Photobiol 2007;83(5):1278–82.
[21] Osterholm MT, Kelley NS, Sommer A, Belongia EA. Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. Lancet Infect Dis 2012;12(1):36–44.
[22] Bridges CB, Thompson WW, Meltzer MI, et al. Effectiveness and cost–benefit of influenza vaccination of healthy working adults: a randomized controlled trial. JAMA 2000;284(13):1655–63.
[23] Belongia EA, Kieke BA, Donahue JG, et al. Effectiveness of inactivated influenza vaccines varied substantially with antigenic match from the 2004–2005 season to the 2006–2007 season. J Infect Dis 2009;199(2):159–67.
[24] DiazGranados CA, Denis M, Plotkin S. Seasonal influenza vaccine efficacy and its determinants in children and non-elderly adults: a systematic review with meta-analyses of controlled trials. Vaccine 2012;31(1):49–57.
[25] Cumberland P, Shulman CE, Maple PA, et al. Maternal HIV infection and placental malaria reduce transplacental antibody transfer and tetanus antibody levels in newborns in Kenya. J Infect Dis 2007;196(4):550–7.
[26] Brair ME, Brabin BJ, Milligan P, Maxwell S, Hart CA. Reduced transfer of tetanus antibodies with placental malaria. Lancet 1994;343(8891):208–9.
[27] Okoko BJ, Wesuperuma LH, Ota MO, et al. Influence of placental malaria infection and maternal hypergammaglobulinaemia on materno-foetal transfer of measles and tetanus antibodies in a rural west African population. J Health Popul Nutr 2001;19(2):59–65.
[28] Jones CE, Naidoo S, De Beer C, Esser M, Kampmann B, Hesseling AC. Maternal HIV infection and antibody responses against vaccine-preventable diseases in uninfected infants. J Am Med Assoc 2011;305(6):576–84.
[29] Gupta A, Mathad JS, Yang W-T, et al. Maternal pneumococcal capsular IgG antibodies and transplacental transfer are low in South Asian HIV-infected mother-infant pairs. Vaccine 2014;32(13):1466–72.
[30] Almeida VDC, Mussi-Pinhata MM, Souza CBSD, et al. Immunogenicity of 23-valent pneumococcal polysaccharide vaccine in HIV-infected pregnant women and kinetics of passively acquired antibodies in young infants. Vaccine 2009;27(29):3856–61.
[31] Abzug MJ, Nachman SA, Muresan P, et al. Safety and immunogenicity of 2009 pH1N1 vaccination in HIV-infected pregnant women. Clin Infect Dis 2013;56(10):1488–97.
[32] Kallenberg J, Nguyen A. Report to the programme and policy committee: vaccine investment strategy. Geneva: GAVI Alliance; 2013.
[33] UNICEF. Mali—statistics; 2012. ⟨http://www.unicef.org/infobycountry/mali_statistics.html⟩.
[34] UNICEF. Nepal—statistics; 2012. ⟨http://origin-www.unicef.org/infobycountry/nepal_nepal_statistics.html⟩.
[35] UNICEF. South Africa—statistics; 2012. ⟨http://origin-www.unicef.org/infobycountry/southafrica_statistics.html⟩.
[36] F D-S, Laski L, Mason E. A universal pathway—a woman's right to health. The State of World's Midwifery; 2014. ⟨http://www.unfpa.org/sites/default/files/pub-pdf/EN_SoWMy2014_complete.pdf⟩.
[37] WHO. Probability of dying per 1000 live births. In: Data by country; 2012. ⟨http://apps.who.int/gho/data/node.main.ChildMort-2?lang=en⟩.